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The metathoracic spiracles in some ants and wasps (Hymenoptera: Formicidae; Vespidae)

Метаторакальные дыхальца некоторых муравьев и ос (Hymenoptera: Formicidae; Vespidae)

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KEYWORDS: Aculeata, Comparative anatomy, mesosoma, mesopleuron, metapleuron, spiracular mechanism.

КЛЮЧЕВЫЕ СЛОВА: Aculeata, сравнительная морфология, мезосома, мезоплеврон, метаплеврон, механизм дыхальца.

ABSTRACT. The second thoracic spiracle and the adjacent skeleton were studied in twenty-two ant species from nine Formicidae subfamilies (Formicinae, Dolichoderinae, Myrmeciinae, Paraponerinae, Ponerinae, Ectatomminae, Myrmicinae, Dorylinae and Ecitoninae) and two wasp species from Vespidae (*Vespa crabro* L., 1758 and *Dolichovespula* sp.). Dissections followed by scanning electron microscopy or photography revealed that the studied alate forms with concealed spiracles from both Aculeata families possess the spiracular occlusor muscle in addition to the supramesopleural sclerite concealing the true spiracle. There is assumed to be a homologous relationship between the re-discovered spiracular occlusor muscle of Aculeata and the corresponding muscle of the metathoracic spiracle in Symphyta. The comparison of winged Formicidae and ant workers with concealed spiracles demonstrated the similarities between them both on the position of plates covering the metathoracic spiracles (i.e. supramesopleural sclerite and spiracular lobe, respectively), and on the position of the spiracular occlusor muscles. Accordingly, the spiracular lobe of worker ants of Formicinae, Dolichoderinae, Myrmeciinae, Paraponerinae, Ponerinae and Ectatomminae was determined to be a derivative of the supramesopleural sclerite that is essential to their winged females. At the same time the exposed type of spiracles observed in studied Myrmicinae, Dorylinae and Ecitoninae ant workers corresponds with the absence of the supramesopleural sclerite in the alate forms.

РЕЗЮМЕ. Второе грудное дыхальце и примыкающий к нему скелет были изучены у 22 видов муравьёв из 9 подсемейств Formicidae (Formicinae, Dolichoderinae, Myrmeciinae, Paraponerinae, Ponerinae, Ectatomminae, Myrmicinae, Dorylinae и Ecitoninae), а также у двух видов ос из Vespidae (*Vespa crabro* L. 1758 and *Dolichovespula* sp.). Препаровка с последующей сканирующей электронной микроскопией либо фотосъёмкой показали, что изученные крылатые формы со скрытыми дыхальцами из обоих семейств Aculeata имеют запирающую дыхальцевую мышцу в дополнение к супрамезоплевральному склериту, скрывающему истинное дыхальце. Предполагается гомология между повторно обнаруженной дыхальцевой мышцей Aculeata и соответствующей мышцей метаторакального дыхальца Symphyta. Сравнение крылатых Formicidae и рабочих муравьев со скрытыми дыхальцами продемонстрировало сходство между ними как по положению пластинок, покрывающих дыхальца (т.е. супрамезоплеврального склерита и дыхальцевой лопасти, соответственно), так и по положению дыхальцевых мышц. Поэтому дыхальцевую лопасть рабочих муравьёв Formicinae, Dolichoderinae, Myrmeciinae, Paraponerinae, Ponerinae, Ectatomminae следует считать дериватом супрамезоплеврального склерита, присущего крылатым самкам. В свою очередь, открытый тип дыхалец у рабочих муравьёв Myrmicinae, Dorylinae и Ecitoninae согласуется с отсутствием супрамезоплеврального склерита у крылатых форм из тех же подсемейств.

Introduction

As the metathoracic spiracles are inconspicuous or concealed, it is difficult to investigate their morphology and phylogeny in aculeate hymenoptera. Nevertheless, their position is one of the important landmarks on the hymenoptera thorax [Reid, 1941]. In winged forms, the second thoracic spiracles are concealed beneath the bases of the hind wings; in wingless forms, the original position of the spiracle is in the membrane between the meso- and metapleuron. According to Reid, in many forms the second spiracle is at the dorsal termination of the mesometapleural suture, and with very few exceptions it is always at the junction of the dorsum and pleura.

Meanwhile et Duncan [1939] noted that second thoracic spiracle of vespine wasps is covered by a small sclerite.

He referred to it as the spiracular peritreme, but subsequent comparison with other Hymenoptera suggested that it is a real sclerite [Tonapi, 1958]. Primarily it was attributed to the posterodorsal corner of the mesopleuron [Duncan, 1939; Tonapi, 1958]. However, currently, based on data of symphytans, modern authors referred to sclerite as certain intersegmentalia that were affiliated with the mesopleuron [Vilhelmsen, 2000; Vilhelmsen et al., 2010]. Besides vespine wasps, the sclerite that conceals or partly covers the second thoracic spiracle was detected in some other taxa of Aculeata. As a result, it acquired a variety of denominations: **collar** [Tonapi, 1958]; **epimeral lobe** [Richards, 1977; Yoshimura, Fisher, 2007]; **basalar lobe** [Bohart, Menke, 1976; Deyrup, Cover, 2004; MacGown et al., 2014], **supramesopleural sclerite** [Vilhelmsen et al., 2010], **spiracular sclerite** [Boudinot, 2015].

The mechanism of the second thoracic spiracle in Aculeata representatives remains uncertain although many researchers have studied it. Most agree with certain similarities between the second spiracle and the first one, in which the aperture is regulated by a single occlusor muscle. The principal disagreement concerns the existence of the corresponding muscle. For example, there is an occlusor muscle of the second thoracic spiracle in several ant species [Nasonov, 1889; Tonapi, 1958, 1959; Delye, 1965]. Duncan [1939], Tonapi [1958] and Richards [1977] noted the presence of the occlusor muscle in vespine wasps while Snodgrass [1925] declared its absence in honeybees. In addition to above mentioned aculeates Tonapi [1958] studied spiracular structures in Symphyta species. Most of his conclusions relating to the presence of an occlusor muscle of the second thoracic spiracle in symphytans were corroborated [Vilhelmsen, 2000], but the muscle's existence in aculeates requires additional studies. The main problem is that it is such a minute muscle and is therefore difficult to detect. Moreover, sometimes it "is not possible to discern between concealment and absence of the spiracle because this fragile structure was often destroyed during dissection" [Vilhelmsen et al., 2010: 72].

Since the attributes of the metathoracic spiracle as well as the state of its associated sclerite are important for interpretation of Aculeata phylogeny, it was further evaluated in this study. It was necessary to demonstrate the adjacent skeleton and the components of the spiracle at least in representatives of the two families Vespidae and Formicidae. The number of species in Formicidae was greater for the following reasons. Most Formicidae species have winged sex individuals (males and females) and wingless workers (non-reproductive females), that markedly differed in thorax morphology. Moreover the thorax structure varies between Formicidae subfamilies. Therefore it was necessary to study the representatives of winged and wingless forms of different taxa. Meanwhile recent work devoted to analysis of Formicidae morphology and phylogeny included data only about the external state of the metathoracic spiracles — concealed or exposed — in ant workers [Keller, 2011]. The similar characteristics of Formicidae males, such as the presence or absence of sclerite concealing the second thoracic spiracle, were analysed in the course of compiling the attributes of species, genera and subfamilies [Yoshimura, Fisher, 2007, 2012; Boudinot, 2015]. Information about the status of this trait in females is published infrequently [Ogata 1987, 1991]. However the internal structure of the metathoracic spiracle in Formicidae species is not mentioned in modern publications.

Materials and methods

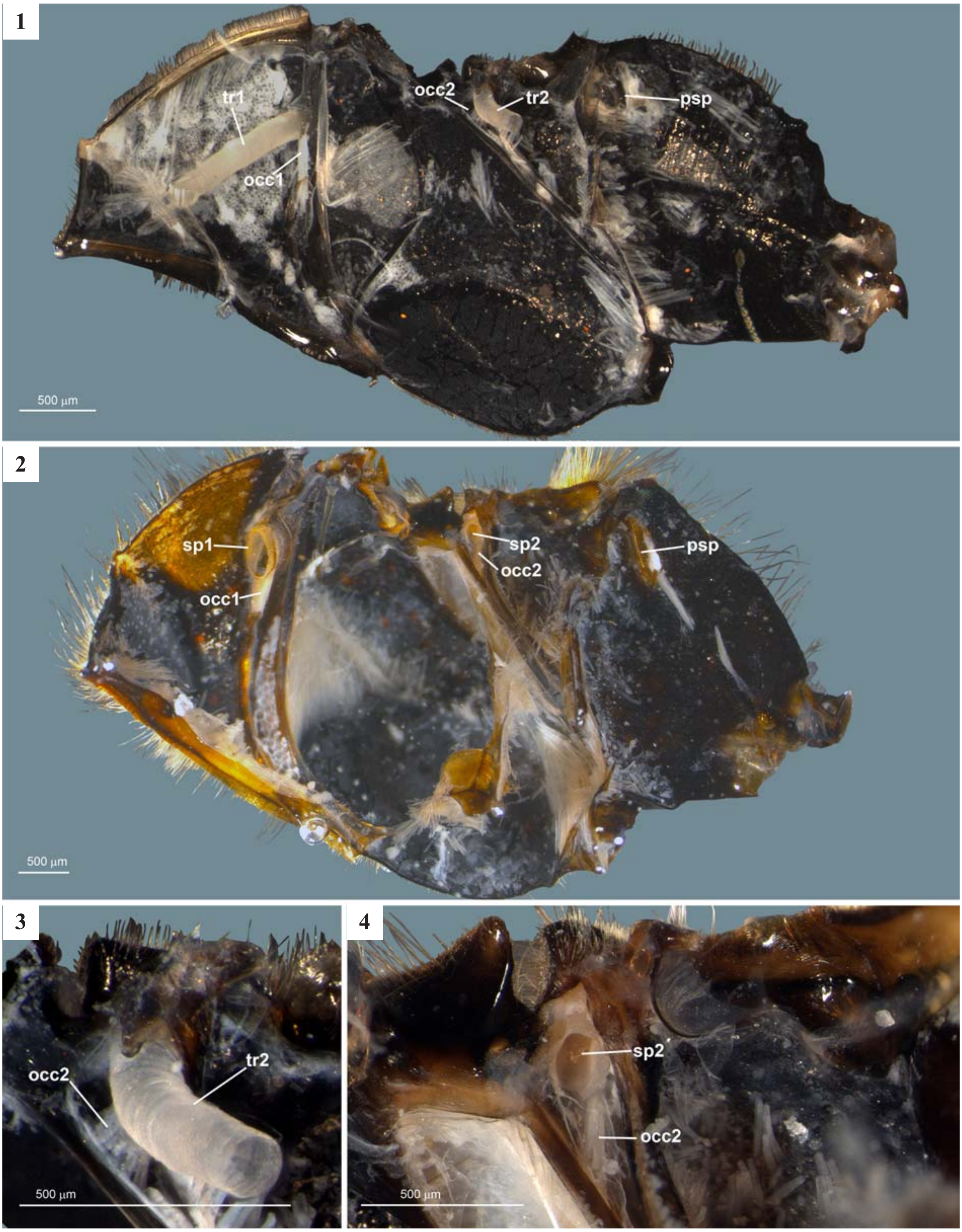
The examined material included two wasp species from Vespidae (Vespinae), *Vespa crabro* Linnaeus, 1758 and *Dolichovespula* sp., and twenty-three ant species from nine subfamilies of Formicidae: Formicinae, Dolichoderinae, Myrmeciinae, Paraponerinae, Ponerinae, Ectatomminae, Myrmicinae, Dorylinae and Ecitoninae (Table). Detailed information about specimens is presented in Appendix 1.

Table. List of examined Formicidae species
Таблица. Список изученных видов Formicidae

Subfamily	Species
Dolichoderinae	*Azteca* sp.
	Dolichoderus attelaboides (Fabricius, 1775)
Dorylinae	*Dorylus orientalis* Westwood, 1835
Ecitoninae	*Eciton burchellii* (Westwood, 1842)
Ectatomminae	*Ectatomma tuberculatum* (Olivier, 1792)
Formicinae	*Ñamponotus* (*Tanaemyrmex*) sp.
	Ñamponotus herculeanus (Linnaeus, 1758)
	Formica aquilonia Yarrow, 1955
	Formica exsecta Nylander, 1846
	Formica lugubris Zetterstedt, 1838
	Lasius niger (Linnaeus, 1758)
Myrmeciinae	*Myrmecia* sp.
	Myrmecia piriformis Smith, F., 1858
Myrmicinae	*Aphaenogaster gibbosa* (Latreille, 1798)
	Atta sexdens (Linnaeus, 1758)
	Manica rubida (Latreille, 1802)
	Messor denticulatus Santschi, 1927
	Myrmica lobicornis Nylander, 1846
	Myrmica ruginodis Nylander, 1846
	Pogonomyrmex badius (Latreille, 1802)
Paraponerinae	*Paraponera clavata* (Fabricius, 1775)
Ponerinae	*Diacamma geometricum* Smith F., 1857
Ponerinae	*Dinoponera gigantean* (Perty, 1833)

Most of the material for the present study was preserved in 70% ethanol. Dried material was used in some instances. The dissections were carried out with tools for ophthalmic microsurgery (spring scissors, blade holder and forceps), pieces of razor blades and hair, and entomological pins. Prior to dissecting the mesosoma, the head, legs, wings and metasoma were removed. Dissection of wet mounts was executed in Petri dishes (35×10 mm) filled with water under a stereomicroscope Olympus SZ61. Dissected specimens were placed in 70% ethanol. Photographs of wet specimens were taken using a Leica M165C stereomicroscope with a Leica DFC425 digital camera, Z-stacked using Helicon Focus 4.10 at PIN RAS. The preparations for scanning electron microscopy (SEM)

were transferred from 70 to 99.9% ethanol in an ethanol series and critical-point dried. The preparations were mounted on SEM stubs, sputter-coated with gold and photographed using Tescan Vega TS5130MM scanning

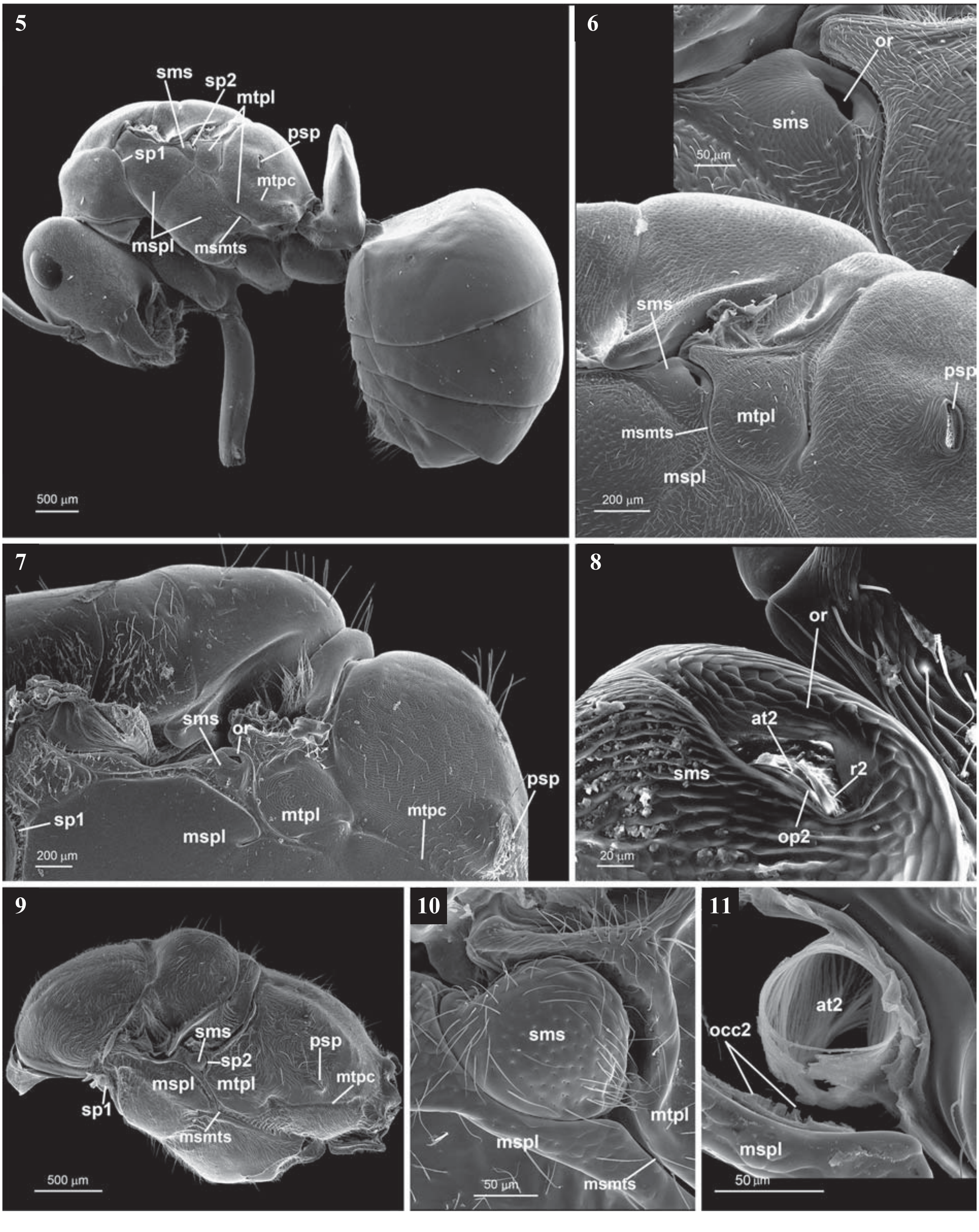


Figs 1–4. Mesosoma and its spiracles in Vespidae, internal lateral view, anterior to the left: 1, 3 — *Dolichovespula* sp., worker; 2, 4 — *Vespa crabro,* worker. Abbreviations in Appendix 2.

Рис. 1–4. Мезозосома и её дыхальца у Vespidae, латеральный вид изнутри, передний конец слева: 1, 3 — *Dolichovespula* sp., рабочая особь; 2, 4 — *Vespa crabro,* рабочая особь. Обозначения в Приложении 2.

electron microscope at IPEE RAS. All images were edited in Adobe Photoshop CS2.

In general, terminology follows The Hymenoptera Glossary represented at the site of the Hymenoptera



Figs 5–11. Mesosoma and its spiracles in alate Formicidae, lateral view, anterior to the left: 5–6 — *Formica aquilonia* (Formicinae), female; 7–8 — *Camponotus* sp. (Formicinae), female; 9–11 — *Diacamma geometricum* (Ponerinae), male. Abbreviations in Appendix 2.

Рис. 5–11. Мезозосома и её дыхальца у крылатых Formicidae, латеральный вид изнутри, передний конец слева: 5–6 — *Formica aquilonia* (Formicinae), самка; 7–8 — *Camponotus* sp. (Formicinae), самка; 9–11 — *Diacamma geometricum* (Ponerinae), самец. Обозначения в Приложении 2.

Anatomy Ontology project [Yoder et al., 2010]. As some terms have several interpretations, the chosen definitions are below:

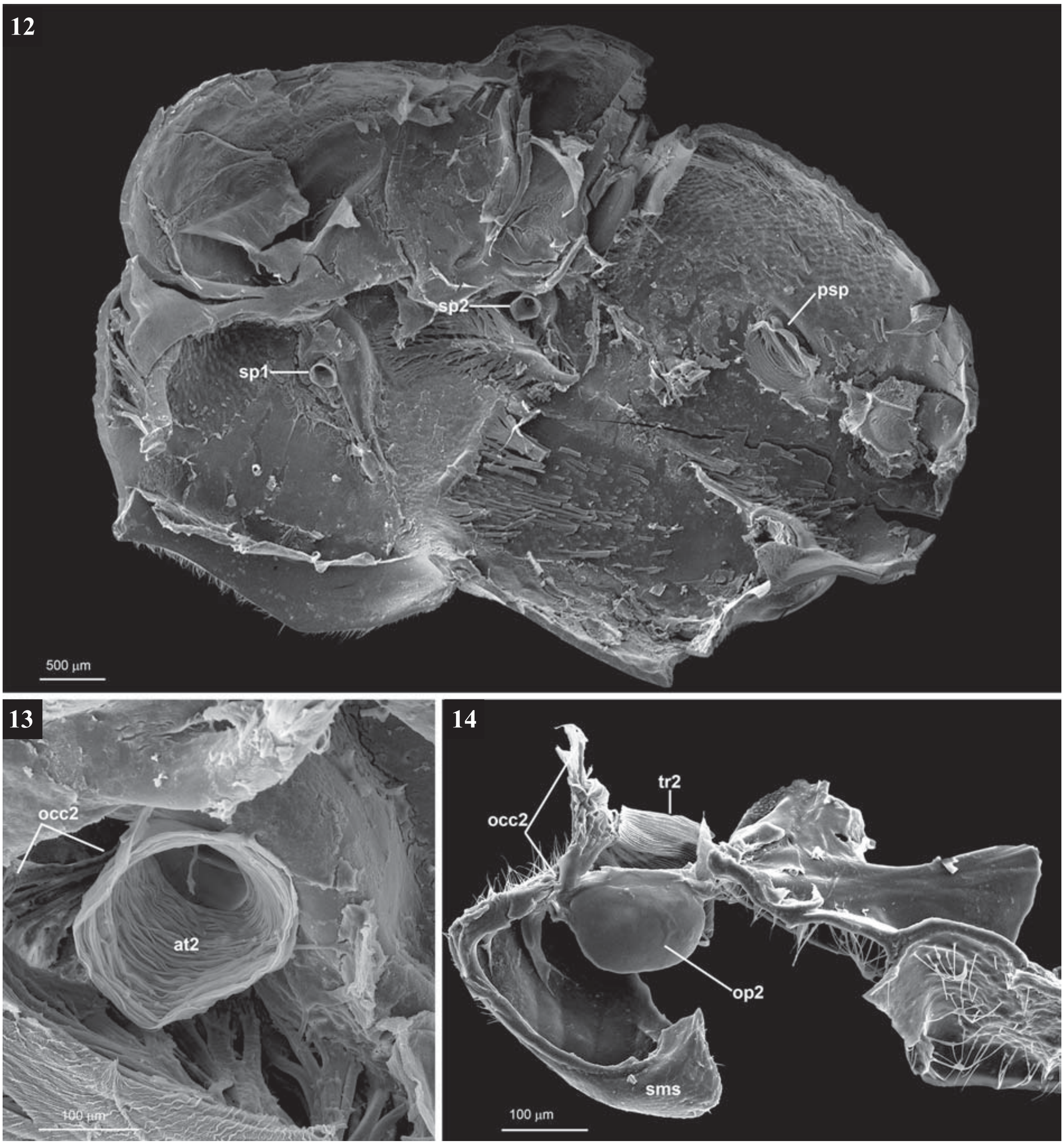
mesopleuron — the area that is located lateral to the mesodiscrimen;

metapleuron — the area of the metapectal-propodeal complex that is located lateral to the metadiscrimen;

mesometapleural suture — the suture that is located between the mesopleuron and the metapleuron;

metapleural carina — the carina that delimits the metapleuron dorsally from the propodeum, extends from just ventral to the metapleural arm to the meta-coxal articulation and passes anteroventral to the propodeal spiracle.

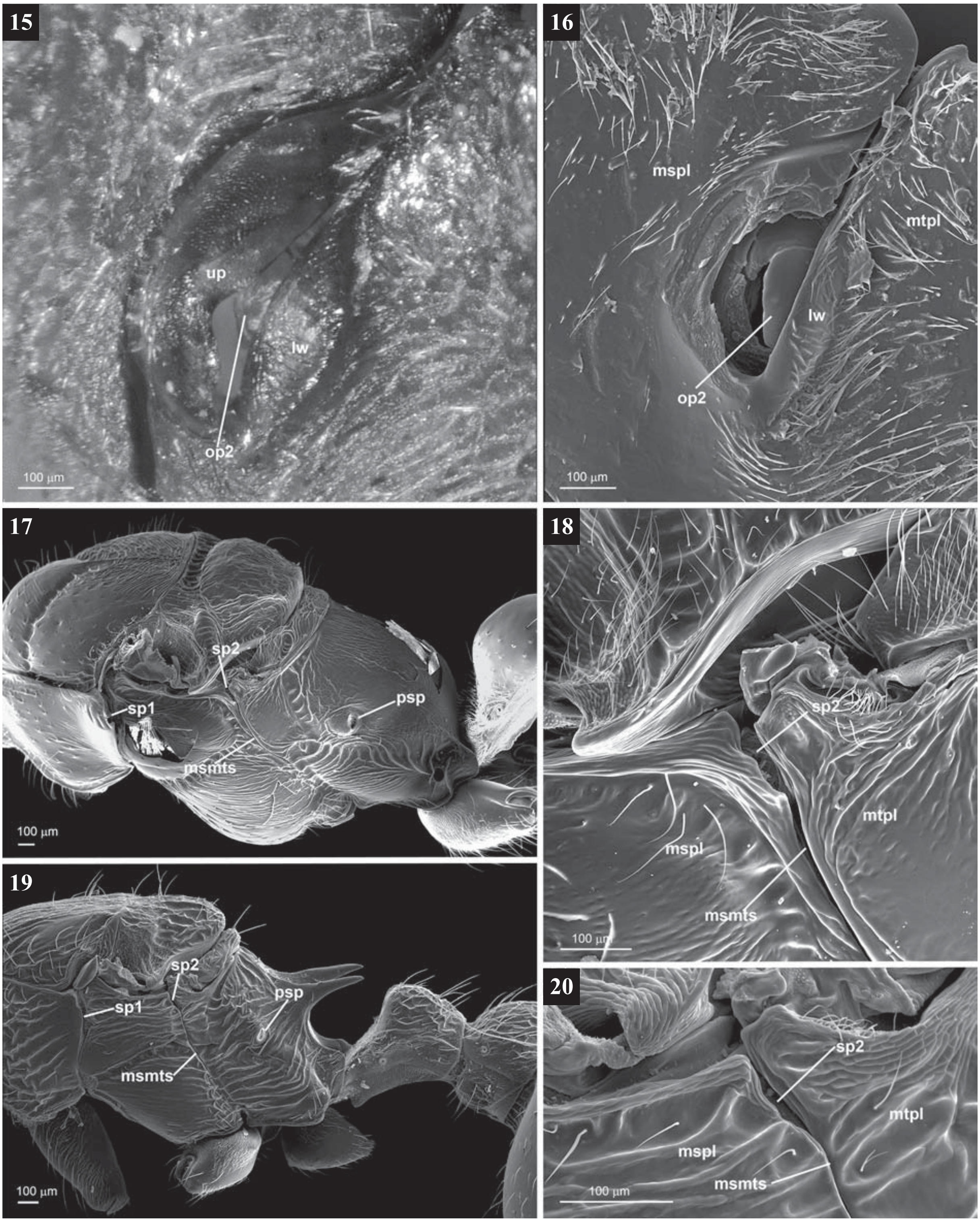
Such terms like **operculum, arm**, and **rim** follow Snodgrass [1925], who used them to differentiate com-



Figs 12–14. Mesosoma and its spiracles in alate ants and wasps: 12–13 — *Paraponera clavata* (Formicidae, Paraponerinae), female internal lateral view, anterior to the left; 14 — *Dolichovespula* sp. (Vespidae: Vespinae), worker, dorsal view, anterior to the left: the left metathoracic spiracle with inside-out supramesopleural sclerite. Abbreviations in Appendix 2.

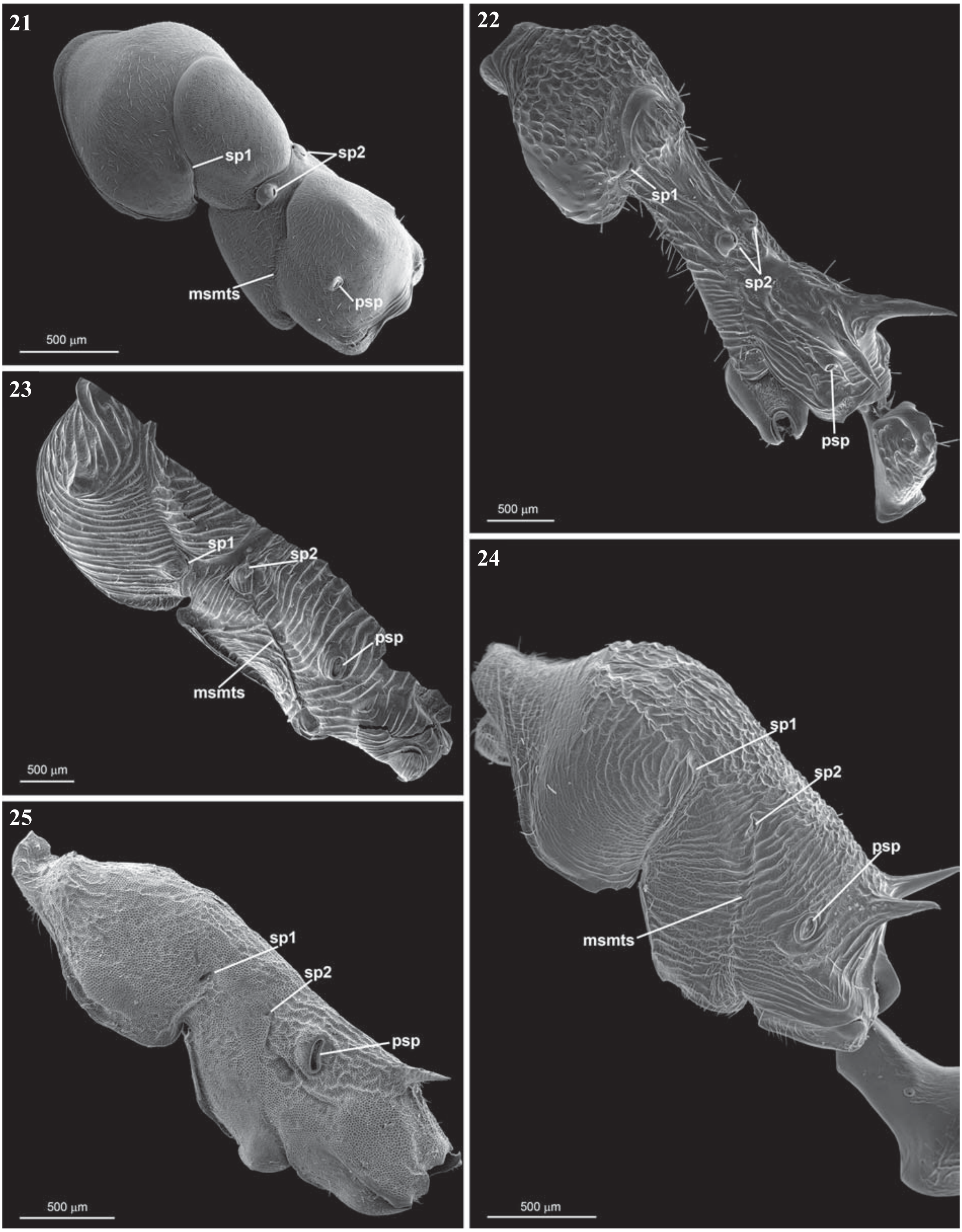
Рис. 12–14. Мезозосома и её дыхальца у крылатых муравьёв и ос: 12–13 — *Paraponera clavata* (Formicidae: Paraponerinae), самка, латеральный вид изнутри, передний конец слева; 14 — *Dolichovespula* sp. (Vespidae:Vespinae), рабочая особь, сверху, передний конец слева, левое метаторакальное дыхальце с вывернутым наружу супрамезоплевральным склеритом. Обозначения в Приложении 2.

ponents of the first thoracic spiracle. **Spiracular valves** are undifferentiated closing plates of the entrance to the second spiracle. Other terms which are absent in the Glossary are given with references to the publications.



Figs 15–20. Mesosoma and its spiracles in alate Formicidae, lateral view, anterior to the left: 15 — left metathoracic spiracle of *Dorylus orientalis* (Dorylinae), male; 16 — the same, partly removed projections of meso- and metapleuron; 17–18 — *Manica rubida* (Myrmicinae), male; 19–20 — *Myrmica ruginodis*, female (Myrmicinae). Abbreviations in Appendix 2.

Рис. 15–20. Мезозосома и её дыхальца у крылатых Formicidae, сбоку, передний конец слева: 15 — левое метаторакальное дыхальце *Dorylus orientalis* (Dorylinae), самец; 16 — то же, частично удалены лопасти мезо- и метаплеврона; 17–18 — *Manica rubida* (Myrmicinae), самец; 19–20 — *Myrmica ruginodis*, самка (Myrmicinae). Обозначения в Приложении 2.



Figs 21–25. Mesosoma and its spiracles in Formicidae workers, lateral-dorsal view, anterior to the left: 21 — *Formica exsecta* (Formicinae); 22 — *Dolichoderus attelaboides* (Dolichoderinae); 23 — *Ectatomma tuberculatum* (Ectatomminae); 24 — *Pogonomyrmex badius* (Myrmicinae); 25 — *Eciton burchellii* (Ecitoninae). Abbreviations in Appendix 2.

Рис. 21–25. Мезозосома и её дыхальца у рабочих Formicidae, сверху-сбоку, передний конец слева: 21 — *Formica exsecta* (Formicinae); 22 — *Dolichoderus attelaboides* (Dolichoderinae); 23 — *Ectatomma tuberculatum* (Ectatomminae); 24 — *Pogonomyrmex badius* (Myrmicinae); 25 — *Eciton burchellii* (Ecitoninae). Обозначения в Приложении 2.

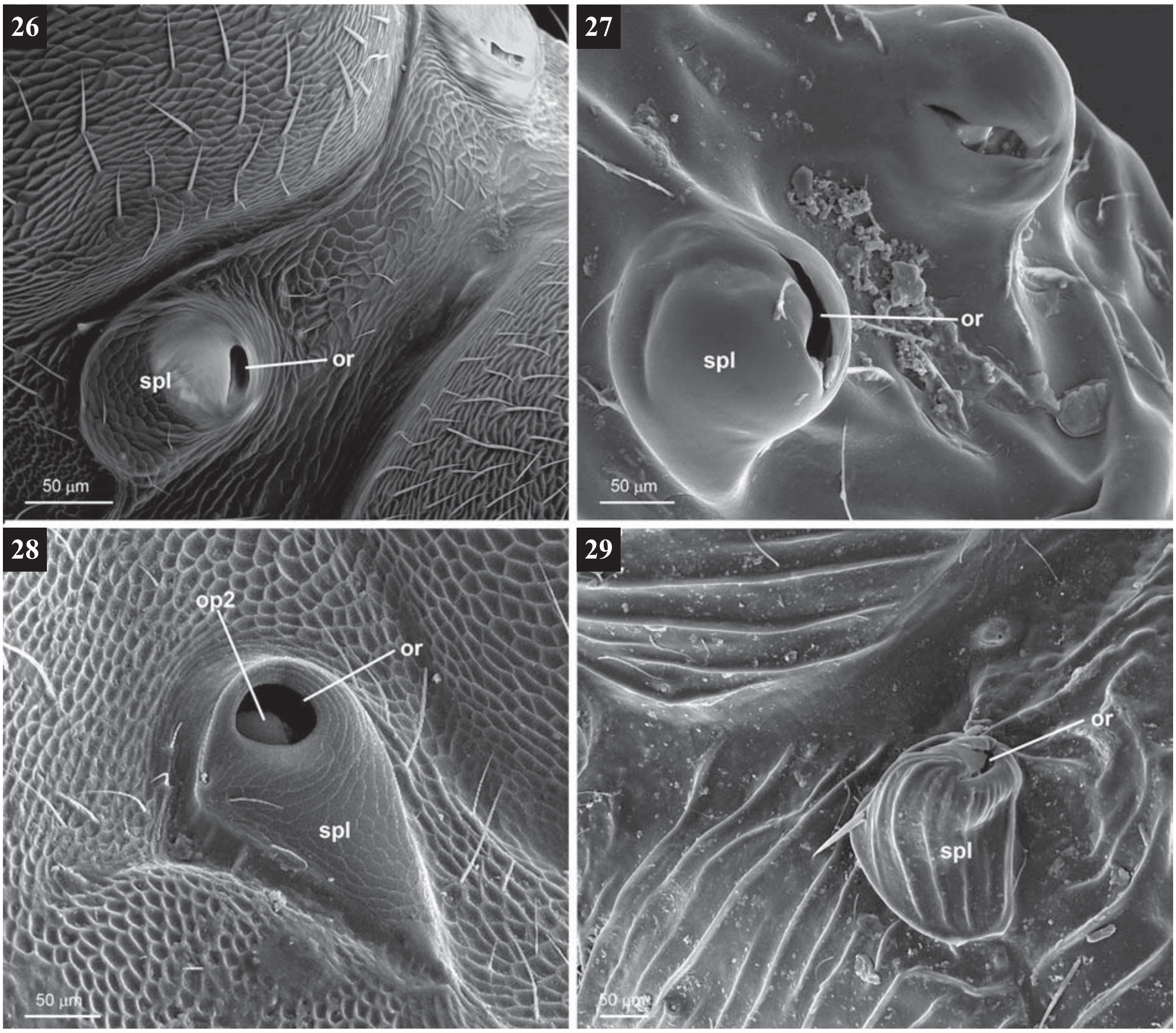
Results

Metathoracic spiracles of winged forms
Figs 1–20.

The supramesopleural sclerite is clearly expressed in both studied wasp species. A spiracle atrium furnished with a blocking hemispheric plate (operculum) is located in the membranous area beneath the sclerite. Although the diameter of the appropriate trachea is less than in the mesothoracic spiracle (Figs 1–2), the aperture of the spiracular atrium is also regulated by the occlusor muscle. The origin of that small, fan-shaped muscle, consisting of a few fibres, lies on the upper posterior edge of the mesopleuron while the operculum is the site of its attachment (Figs 3–4; 14).

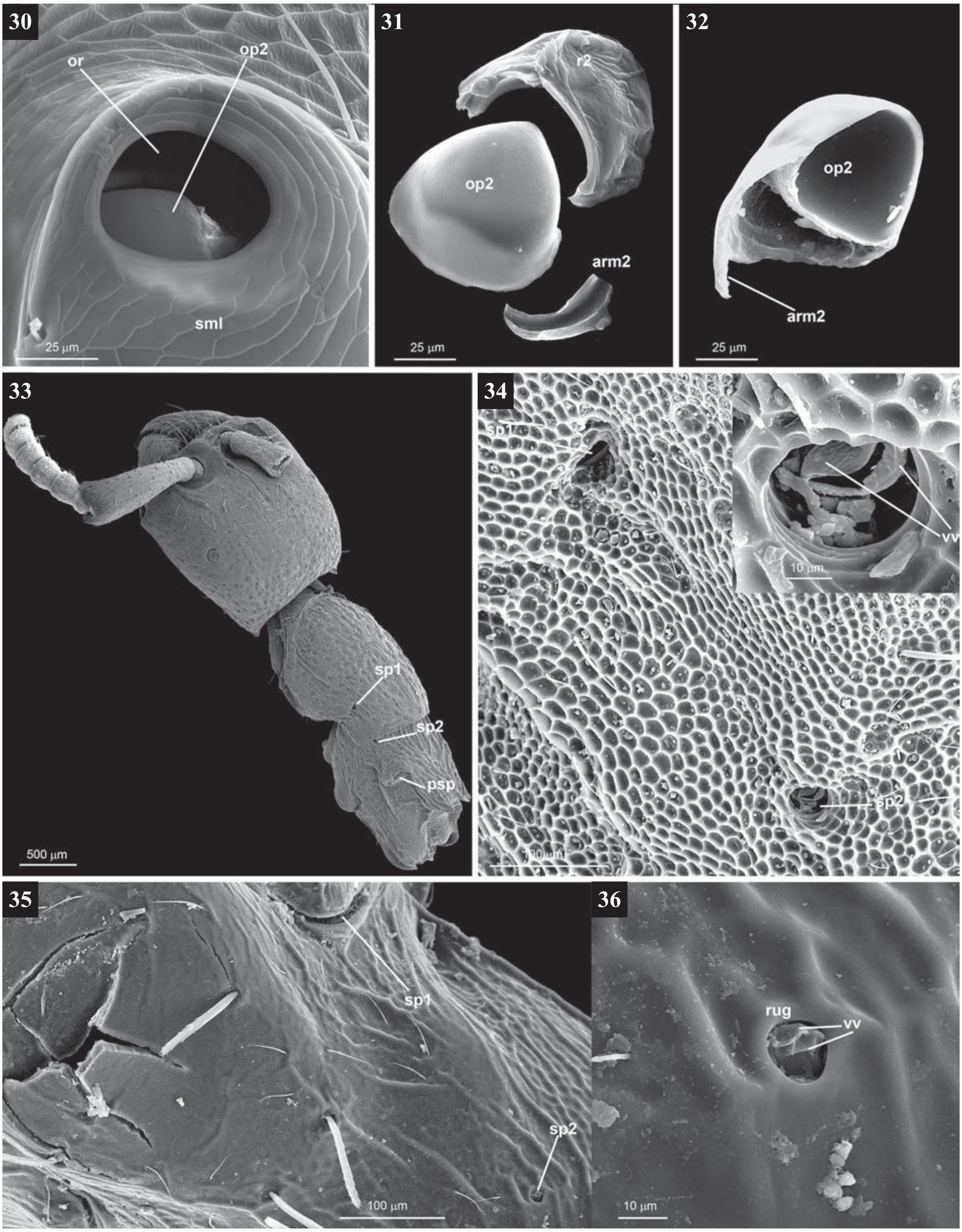
A differentiated supramesopleural sclerite was observed in studied males and females of Formicinae, Dolichoderinae, Paraponerinae and Ponerinae. Its shape varies from triangular to oval (Figs 5–11). In females of *Formica aquilonia*, *Camponotus herculeanus* and *Camponotus* sp., a triangular plate firmly fixed between the meso- and metapleuron has an orifice just opposite the spiracle opening (Figs 6–7) that is evident under high magnification (Fig. 8). As in vespine wasps, the spiracular mechanism of male *Diacamma geometricum* (Figs 9–11) and female *Paraponera clavata* (Figs 12–13) includes an operculum, a lower rim and a fan-shaped occlusor muscle, whose origin site is on the mesopleuron near the border with the supramesopleural sclerite.

The male of *Dorylus orientalis* has no distinct supramesopleural sclerite, but the second thoracic spiracle possessing the operculum is protected by two opposite outgrowths: an upper "lobe" related to the mesopleuron and a lower one related to the metapleuron (Figs 15–16).



Figs 26–29. Metathoracic spiracles in Formicidae workers, lateral-dorsal view, anterior to the left: 26 — *Formica exsecta* (Formicinae); 27 — *Dolichoderus attelaboides* (Dolichoderinae); 28 — *Azteca* sp. (Dolichoderinae); 29 — *Ectatomma tuberculatum* (Ectatomminae). Abbreviations in Appendix 2.

Рис. 26–29. Метаторакальные дыхальца у рабочих Formicidae, сверху-сбоку, передний конец слева: 26 — *Formica exsecta* (Formicinae); 27 — *Dolichoderus attelaboides* (Dolichoderinae); 28 — *Azteca* sp. (Dolichoderinae); 29 — *Ectatomma tuberculatum* (Ectatomminae). Обозначения в Приложении 2.



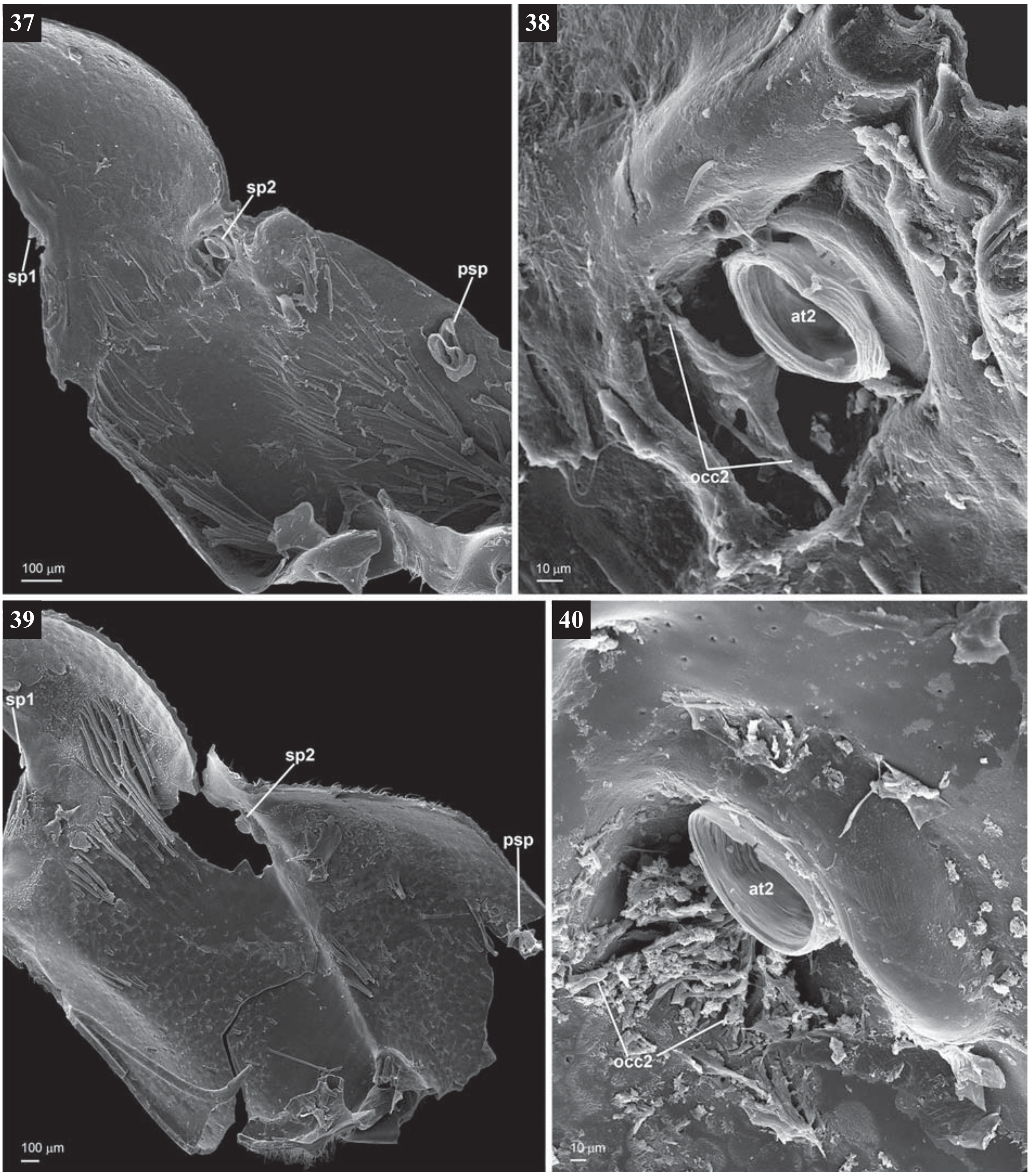
Figs 30–36. Metathoracic spiracles in Formicidae workers: 30–32 — left spiracle of *Azteca* sp. (Dolichoderinae); 30 — lateral-dorsal view, anterior to the left; 31 — skeletal details of the spiracular mechanism; 32 — ventral view of operculum; 33–34 — the left spiracle under two different magnifications, *Eciton burchelli* (Ecitoninae), lateral-dorsal view; 35–36 the right spiracle under low and high magnification, *Aphaenogaster gibbosa* (Myrmicinae), dorsal view, anterior to the left. Abbreviations in Appendix 2.

Рис. 30–36. Метаторакальные дыхальца у рабочих Formicidae: 30–32 — левое дыхальце *Azteca* sp. (Dolichoderinae); 30 — сверху-сбоку, передний конец слева; 31 — скелетные части механизма дыхальца; 32 — снизу, вид на оперкулум; 33–34 — левое дыхальце *Eciton burchelli* (Ecitoninae) при двух разных увеличениях, сверху-сбоку; 35–36 — правое дыхальце при малом и большом увеличении, *Aphaenogaster gibbosa* (Myrmicinae), сверху, передний конец слева. Обозначения в Приложении 2.

The supramesopleural sclerite is also absent in males and females of examined Myrmicinae (Figs 17–20). The second thoracic spiracle lies in a membranous area between the upper opposite edges of the meso- and metapleuron and is hardly visible because usually the pleural edges are are contiguous (Figs 18, 20). As it was possible to discern from a single dry specimen of a male *Atta sexdens* (with pleural edges slid apart), externally this spiracle with two hemispheric valves resembles the second thoracic spiracle of some symphytans.

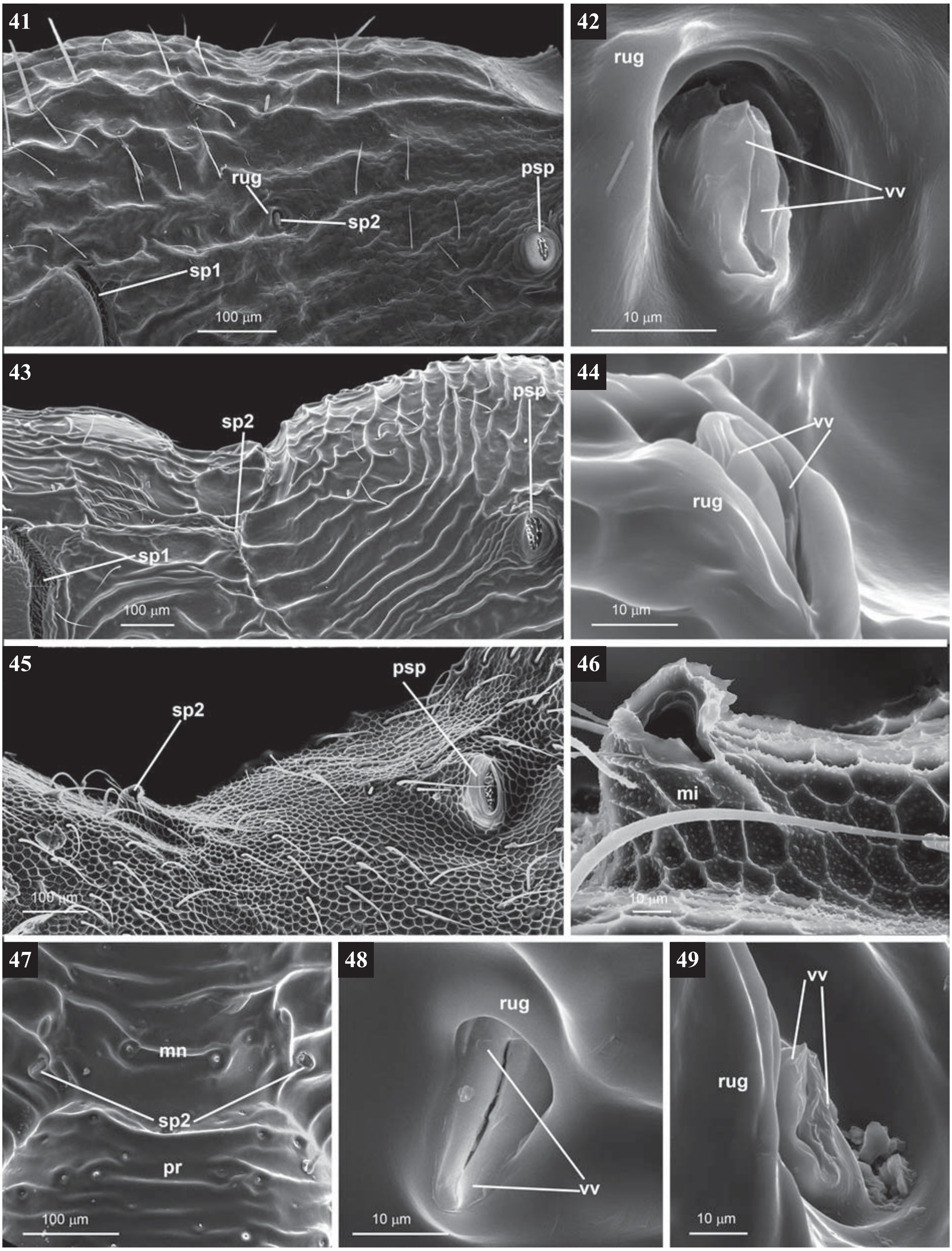
Metathoracic spiracles of ant workers
Figs 21–49.

Crucial changes in the structure of the mesosoma in ant workers due to the absence of flight muscles did not affect the primary position of the second thoracic spira-



Figs 37–40. Metathoracic spiracles in Formicidae workers, internal lateral view, anterior to the left: 37–38 — *Formica aquilonia* (Formicinae); 39–40 — *Myrmecia pyriformis* (Myrmeciinae). Abbreviations in Appendix 2.

Рис. 37–40. Метаторакальные дыхальца у рабочих Formicidae, изнутри-сбоку, передний конец слева: 37–38 — *Formica aquilonia* (Formicinae); 39–40 — *Myrmecia pyriformis* (Myrmeciinae). Обозначения в Приложении 2.



Figs 41–49. Metathoracic spiracles in ant workers of Myrmicinae: 41–42 — *Myrmica lobicornis*; 43–44 — *Manica rubida*; 45–46 — *Atta sexdens*; 47–48 — *Messor denticulatus*; 49 — *Pogonomyrmex badius*; 41– 46 — lateral view, anterior to the left; 47–48 — dorsal view; 49 — lateral-dorsal view. Abbreviations in Appendix 2.

Рис. 41–49. Метаторакальные дыхальца у рабочих муравьёв Myrmicinae: 41–42 — *Myrmica lobicornis*; 43–44 — *Manica rubida*; 45–46 — *Atta sexdens*; 47–48 — *Messor denticulatus*; 49 — *Pogonomyrmex badius*; 41– 46 — сбоку, передний конец слева; 47–48 — сверху; 49 — сбоку-сверху. Обозначения в Приложении 2.

cles. As in alate forms each one is located at an upper point of the mesometapleural junction (above the mesometapleural pit) despite the fact that an appropriate suture between the meso- and metapleuron is often scarcely detected or is absent (Figs 21–25).

All examined second thoracic spiracles of workers of Formicinae, Dolichoderinae, Ectatomminae, Ponerinae, Paraponerinae and Myrmeciinae were positioned under differentiated plates [spiracular lobes — by Keller, 2011]. The shape of the plate, forming a protective chamber over the true spiracle, may be markedly different, but the presence of either an orifice or gap is constant (Figs 26–29). The spiracular atrium opens into the chamber and has a blocking operculum (Figs 28, 30–32) with an attached occlusor muscle that regulates the atrium aperture. A small fan-shaped muscle consists of several fibres that originate on the border of plate and the mesothorax (Figs 37–40). As in alate forms, the composition of skeletal structures forming the true spiracle includes the operculum with the arm (site of muscle insertion) and the rim (Figs 31–32). The operculum and immovable rim jointly encircle the atrium entrance while muscle contraction regulates the split between them by moving the operculum in the same way as in the mesothoracic spiracle. The origin site of the spiracular muscle as well as the close association between the plate and metathoracic spiracle indicates that the spiracular lobe of workers is derived from the supramesopleural sclerite of alate females.

In contrast to the above mentioned subfamilies the Myrmicinae workers have so-called exposed metathoracic spiracles. Such a spiracle is hardly detected even in major workers of *Pogonomyrmex* Mayr, 1868 and *Messor* Forel, 1890. The spiracular atrium is located at a minute cuticular orifice surrounded by rugae, but in most cases it becomes distinctly visible under high magnification. It is equipped with two valves that are weakly sclerotised in *Myrmica lobicornis* (Figs 41–42), *Pogonomyrmex badius* (Figs 24, 49) or *Aphaenogaster gibbosa* (Figs 35–36), but are more heavily sclerotised in *Manica rubida* (Figs 43–44) and *Messor denticulatus* (Figs 47–48). A small distinctive micro-cone with an apical foramen conceals the spiracle atrium at the cuticle of *Atta sexdens* (Figs 45–46). In all dissected specimens of Myrmicinae workers the spiracular muscle could not be found. The same problem concerns workers of *Eciton burchellii* (Ecitoninae) that also have an exposed metathoracic spiracle, but the spiracular entrance is located deeper than in Myrmicinae species (at the bottom of a short tubule) and is equipped with two markedly sclerotised valves: the larger one somewhat resembles the operculum in its shape and position (Figs 33–34).

Discussion and conclusions

Despite the use of data on the state of metathoracic spiracles in modern taxonomic and phylogenetic studies on Formicidae, the homology between the supramesopleural sclerite of alate forms and the spiracular lobe of workers was not established until now. Meanwhile, the comparison of the external mesosomal structure between three types of ant females — alate, ergatoid queens and workers in *Myrmecia* F., 1804 and *Paltothyreus* Mayr, 1862 ants, executed by Emery [1900], pointed directly to such a possibility.

Nasonov [1889] was the first who noted the presence of muscle in the second thoracic spiracle of the *Lasius flavus* ant worker. Subsequently Tonapi [1958] detected this muscle in spiracles of *L. fuliginosus, L.niger, Formica fusca* ants and also in males of *Dorylus labiatus* [Tonapi, 1959]. But his images of the spiracular mechanism were extremely schematic especially in comparison with a more recent description executed by Delye [1965] on the *Camponotus compressus* worker. Delye noted that the muscle originates on the border of the plate covering the second spiracle and also noted that the plate forms a chamber with a small orifice above the true spiracle.

The present study demonstrated that the occlusor muscle attached to the operculum is really present in the metathoracic spiracle at least in studied species of Vespinae wasps and also in the representatives of Formicinae, Dolichoderinae, Myrmeciinae, Ponerinae, Paraponerinae and Ectatomminae ants. The muscle originates at the border of the mesopleuron and supramesopleural sclerite (in females and males) or at the border of the mesopleuron and spiracular lobe (ant workers). As both plates (i.e. supramesopleural sclerite and spiracular lobe) occupy a similar position at the mesosoma of winged forms and ant workers, respectively, and the muscles of the second spiracles in winged and wingless ants have a similar site of origin, the homology between the supramesopleural sclerite and spiracular lobe becomes obvious.

According to modern research, the winged Myrmicinae castes (both males and females) have no distinct supramesopleural sclerite [Ogata, 1991; Bouidinet, 2015]. The comparison between three female castes of *Myrmecina nippomica* Wheeler, W.M., 1906 (alate queen, intermorphic queen and worker) undertaken by Miyazaki et al. [2005] revealed in particular that unlike the alate queen, in which the spiracle is hidden between the mesopleuron and metapleuron, the intermorphic queen has an invaginated cuticle around the spiracle opening, so the spiracle lies inside the hole located at the mesopleuron — metapleuron boundary that was not as conspicuous as in alate queens; meanwhile the spiracle opening of the worker is located at the inconspicuous boundary between the two pleurons. As was revealed in the present study regarding alate forms of other Myrmicinae species (*Atta sexdens, Manica rubida, Myrmica ruginodis*), their second spiracle really lies in a membranous area between the upper opposite edges of the meso- and metapleuron, and its valves are scarcely sclerotised. Moreover, unlike the above mentioned subfamilies, the spiracular atrium of studied Myrmicinae workers (*Aphaenogaster gibbosa, Manica rubida*, *Messor denticulatus, Myrmica lobicornis*, *Pogonomyrmex badius*) is provided with two valves, similar in shape and size. These nearly membranous or more strongly sclerotised valves can be observed externally. Therefore, it seems natural that the absence of the

protective sclerite in Myrmicinae females determined the exposed type of metathoracic spiracles in worker ants. Nevertheless, in some cases the spiracle may be concealed. In particular, in *Atta sexdens* workers the cuticular micro-cone covers the true spiracle, thereby becoming a functional analogue of the vestibule chamber formed by the supramesopleural sclerite in workers of the above Formicidae subfamilies.

In Dorylinae males, the true metathoracic spiracle instead of distinct sclerite is protected by two opposite projections of the meso- and metapleural edges. The original description of the *Dorylus labiatus* spiracle supports that at least in males, it is provided with a fan-shaped occlusor muscle attached to the differentiated operculum, and the muscle "takes its origin on an internal arched sclerotised ridge of the postero-dorsal corner of the mesepimeron" [Tonapi, 1959:88]. The state of the second spiracles in workers of the examined Dorylinae and Ecitoninae species are characterised now as exposed because the spiracular lobe is absent [Keller, 2011], but their internal structure needs additional study.

Unfortunately, the methods applied in the current study were insufficient to determine the presence or absence of muscles in exposed metathoracic spiracles of Myrmicinae and Ecitoninae ant workers. However, even the external comparison revealed some inequalities in spiracle structure between both subfamilies. In particular the atrium of the second spiracle in *Eciton burchellii*, in contrast to Myrmicinae species, lies in a short tubule and the spiracular valves are markedly different.

The authors of a comprehensive morphological investigation în the musculature and skeleton of Hymenoptera [Vilhelmsen et al., 2010] suggested that the presence of the supramesopleural sclerite is one of putative autapomorphies of Aculeata. The results of the present study indicated that in addition to sclerite, which conceals the metathoracic spiracle, the representatives of Vespidae as well as species of several Formicidae subfamilies have a spiracular mechanism similar to their mesothoracic spiracle. It is quite probable that the muscle essential to the second thoracic spiracle of Symphyta [Vilhelmsen, 2000; Vilhelmsen et al., 2010] and the re-discovered spiracular muscle in Aculeata are homologous.

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APPENDIX 1

Material examined

Species	Specimens*	Region, date, collector**	det.	Specimen type, dissection	Imaging method ***
Dolichovespula sp.	2 w	Peshki, 05.08.2012, A. Zakharov		wet, diss	SEM, DC
Vespa crabro	1 w	Peshki, 25.07.2012, A. Zakharov	Fedoseeva	wet, diss	DC
Azteca sp.	2 w	Bolivia, 1995, K. Dzerzhinsky		wet, diss	SEM
Dolichoderus attelaboides	2 w	Pukalpa, 1986, A. Zakharov	Dlussky	wet, diss	SEM
Dorylus orientalis	1 m	Cam-Ranh, 04.05.1980, V. Badikov	Dlussky	dry, diss	DC
Eciton burchellii	3 w	Pukalpa, 1986, A. Zakharov	Dlussky	wet, diss	SEM
Ectatomma tuberculatum	2 w	Pukalpa, 1986, A. Zakharov	Dlussky	wet, diss	SEM
Camponotus herculeanus	1 f, 1 w	Pinezhsky NR, 08.08.2010, R. Zakharov	Fedoseeva	wet, diss	—
Camponotus sp.	1 f, 1 w	Lake Louise, 25.06.1988, A. Rasnitsyn		wet, diss	SEM
Formica aquilonia	1 f, 2 w	Pinezhsky NR, 12.08.2012, R. Zakharov	Fedoseeva	wet, diss	SEM
F. exsecta	2 w	Narofominsk, 12.07. 2012, D. Goryunov	Goryunov	wet, diss	SEM
F. lugubris	1 w	Pinezhsky NR, 03.08.2012, R. Zakharov	Fedoseeva	wet, diss	SEM
Lasius niger	3 f	Moscow, 07.07.2014, E. Fedoseeva	Fedoseeva	wet, diss	SEM
Myrmecia pyriformis	1 w	Canberra, 21.06.1978, A. Zakharov	Dlussky	wet, diss	SEM
Myrmecia sp.	2 w	Australia, 1978, A. Zakharov		dry	DC
Aphaenogaster gibbosa	2 w	Muchtumkuli, 06.1983, E. Fedoseeva	Dlussky	dry	SEM
Atta sexdens	2 w	Pukalpa, 1–15.09.1988, A. Suvorov	Dlussky	wet, diss	SEM
A. sexdens	1 m	Pukalpa, 1–15.09.1988, A. Suvorov	Dlussky	dry	DC
Manica rubida	1 m, 1 w	Teberda, 16.08.1958, G.Kurcheva	Armoldi	dry	SEM
Messor denticulatus	2 w	Muhtumkuli, 06. 1981, E. Fedoseeva	Dlussky	wet, diss	SEM
Myrmica lobicornis	1 w	Pinezhsky NR, 10.08.2010, R. Zakharov	Fedoseeva	wet, diss	SEM
M. ruginodis	2 f	Pinezhsky NR, 12.08.2010, R. Zakharov	Fedoseeva	wet, diss	SEM
Pogonomyrmex badius	1 w	Sonoyta, 09.1983, A. Suvorov	Dlussky	dry	SEM
Paraponera clavata	1 f, 1 w	Pukalpa, 1986, A. Zakharov	Dlussky	wet, diss	SEM
Diacamma geometricum	2 m	Nam Cat Tien, 2008, V. Zryanin	Zryanin	wet, diss	SEM
Dinoponera gigantea	1 w	Pukalpa, 1986, A. Zakharov	Dlussky	wet, diss	DC

Abbreviations in the Appendix :

* f — female, m — male, w — worker.

** Canberra — Australia, Canberra; Cam-Ranh — Vietnam, Cam-Ranh; Lake Louise — Canada, Alta: Lake Louise, 150 km W of Calgary; Moscow — Russia, Moscow; Muhtumkuli — Turkmenistan, Balkan Dep., Muchtumkuli (Kara-Kala); Nam Cat Tien — Vietnam, Dong Nai Prov., Nam Cat Tien;Narofominsk — Russia, Moscow Region, Narofominsk District; Peshki — Russia, Moscow Region, 55 km N-W Moscow; Pinezhsky NR — Russia, Arkhangelsk Region, Pinezhsky District, Pinezhsky Nature Reserve; Pukalpa — Peru, Dep. Ukayali, 60 km W Pukalpa; Sonoyta — Mexico, State Sonora, Sonoyta; Teberda — Russia, Karachaevo-Cherkesia Rep., Teberdinsky Zapovednik.

*** SEM — scanning electron microscopy; DC — digital camera.

APPENDIX 2

List of abbreviations used in Figures 1–9

arm2 — operculum arm of the metathoracic spiracle
at2 — atrium of the metathoracic spiracle
lw — projection of the anterior metapleural edge
mi — micro-cone above the metathoracic spiracle
mn — mesonotum
msmts — mesometapleural suture
mspl — mesopleuron
mtpc — metapleural carina
mtpl — metapleuron
occ1 — occlusor muscle of the mesothoracic spiracle
occ2 — occlusor muscle of the metathoracic spiracle
op2 — operculum of the metathoracic spiracle
or — orifice in the supramesopleural sclerite and spiracular lobe
pr — propodeum
psp — propodeal spiracle
r2 — immovable rim of the metathoracic spiracle
rug — cuticular rugae around the metathoracic spiracle
sms — supramesopleural sclerite
sp1 — mesothoracic spiracle
sp2 — metathoracic spiracle
spl — spiracular lobe
tr1 — trachea of the mesothoracic spiracle
tr2 — trachea of the metathoracic spiracle
up — projection of the posterior mesopleuron edge
vv — spiracular valves